



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12N 15/12, 15/85, 5/10, C07K 14/47, A61K 38/17	A2	(11) International Publication Number: WO 97/00319 (43) International Publication Date: 3 January 1997 (03.01.97)
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(54) Title: CHIMERIC LEPTIN FUSED TO IMMUNOGLOBULIN DOMAIN AND USE		
(57) Abstract Chimeric leptin which are proteins comprising leptin or a mutant or a variant thereof fused to a human immunoglobulin domain. One favoured immunoglobulin domain is the human immunoglobulin Fc domain. The chimeric derivatives of leptin have, despite their large molecular size, good pharmacological activity combined with prolonged clearance rates. These derivatives of leptin are therefore indicated to be particularly useful for the treatment or prophylaxis of obesity or diseases and conditions associated with obesity such as atherosclerosis, hypertension and type II diabetes.		

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CHIMERIC LEPTIN FUSED TO IMMUNOGLOBULIN DOMAIN AND USE

The present invention relates to a novel compound being a novel chimeric protein, to a process for the preparation of such a compound, a pharmaceutical composition comprising such a compound and the use of such a compound in medicine, especially for the treatment of obesity and associated diseases.

European Patent Application, Publication number 0 464 533 discloses fusion proteins comprising various portions of the constant region of immunoglobulin molecules together with another human protein or part thereof. European Patent Application, Publication number 0 297 882 discloses fusion proteins comprising various portions of the plasminogen molecule with part of another human protein.

Zhang et al. (Nature: 372, 425 - 432; 1994) describe the positional cloning of a mouse obese gene and its human homologue. The sequence of the Open Reading Frame (ORF) of the mouse gene predicts a polypeptide of 167 amino acids and Zhang et al. predicted the presence of a signal sequence which would lead to the production of a mature protein of 146 residues. The human homologue was disclosed as having a similar size of 146 amino acids for the mature protein. Zhang et al. showed the presence of a primary translation product of approximate size of 18 kilodaltons (kD) with truncation to a 16kD product on addition of microsomal membranes, consistent with the production of a pre-protein and the removal of an N-terminal signal sequence. Zhang et al also disclose the potential use of the human obese gene product (hereinafter 'leptin') in the treatment of obesity.

For effective, practical treatment of obesity a particularly desirable property of an obesity agent is a clearance rate in humans commensurate with patient acceptable treatment regimens, especially regimens for injectable therapies. Zhang et al. do not disclose information relating to the clearance rate of the active molecule in either mouse or humans.

The precise mechanism of action of leptin is currently unknown, however it is considered that in order to provide the observed pharmacological effects, leptin must interact with one or more receptors in the brain.

We have now discovered certain chimeric derivatives of leptin which surprisingly, despite their large molecular size, have good pharmacological activity combined with prolonged clearance rates. These chimeric derivatives of leptin are therefore indicated to be particularly useful for the treatment or prophylaxis of obesity and for the treatment or prophylaxis of diseases and conditions associated with obesity, such as atherosclerosis, hypertension and, especially, Type II diabetes. In particular these compounds are considered to be useful for administration by injection.

These compounds are also considered to be useful in cosmetic treatments for the improvement of body appearance.

Accordingly, the invention provides a chimeric leptin or a chimeric mutant or derivative of leptin.

5 One particular chimeric leptin is a protein comprising leptin or a mutant or variant thereof fused to a human immunoglobulin domain or a mutant or variant thereof.

Suitably, the chimeric protein comprises one human immunoglobulin domain.

Favourably, the human immunoglobulin domain is fused to the C-terminus of leptin.

10 One favoured human immunoglobulin is an human immunoglobulin Fc domain.

An example of a human immunoglobulin Fc domain is an IgG4PE variant in particular IgG4 hinge-CH₂-CH₃PE. Other examples are IgG4, IgG1 and IgG1GT, in particular the hinge-CH₂-CH₃ region in each case.

The term "mutant or variant" used with respect to a particular protein
15 encompasses any molecule such as a truncated or other derivative of the relevant protein which retains substantially the same activity in humans as the relevant protein. Such other derivatives can be prepared by the addition, deletion, substitution, or rearrangement of amino acids or by chemical modifications thereof.

The immunoglobulin may be of any subclass (IgG, IgM, IgA, IgE), but is
20 preferably IgG, such as IgG1, IgG3 or IgG4. The said constant domain(s) or fragment thereof may be derived from the heavy or light chain or both. The invention encompasses mutations in the immunoglobulin component which eliminate undesirable properties of the native immunoglobulin, such as Fc receptor binding and/or introduce desirable properties such as stability. For example, Angal S., King D.J., Bodmer M.W.,
25 Turner A., Lawson A.D.G., Roberts G., Pedley B. and Adair R., Molecular Immunology vol30pp105-108, 1993, describe an IgG4 molecule where residue 241 (Kabat numbering) is altered from serine to proline. This change increases the serum half-life of the IgG4 molecule. Canfield S.M. and Morrison S.L., Journal of Experimental Medicine
vol173pp1483-1491, describe the alteration of residue 248 (Kabat numbering) from
30 leucine to glutamate in IgG3 and from glutamate to leucine in mouse IgG2b. Substitution of leucine for glutamate in the former decreases the affinity of the immunoglobulin molecule concerned for the FcγRI receptor, and substitution of glutamate for leucine in the latter increases the affinity. EP0307434 discloses various mutations including an L to E mutation at residue 248 (Kabat numbering) in IgG.

35 The constant domain(s) or fragment thereof is preferably the whole or a substantial part of the constant region of the heavy chain of human IgG. The IgG

component suitably comprises the CH2 and CH3 domains and the hinge region including cysteine residues contributing to inter-heavy chain disulphide bonding.

For example when the IgG component is derived from IgG4 it includes cysteine residues 8 and 11 of the IgG4 hinge region (Pinck J.R. and Milstein C., Nature
5 vol216pp941-942, 1967). Preferably the IgG4 component consists of amino acids corresponding to residues 1-12 of the hinge, 1-110 of CH2 and 1-107 of CH3 of IgG4 described by Ellison J., Buxbaum J. and Hood L., DNA vol1pp11-18, 1981. In one example of a suitable mutation in IgG4, residue 10 of the hinge (residue 241, Kabat numbering) is altered from serine (S) in the wild type to proline (P) and residue 5 of CH2
10 (residue 248, Kabat numbering) is altered from leucine (L) in the wild type to glutamate (E).

DNA polymers which encode mutants or variants of the human immunoglobulin may be prepared by site-directed mutagenesis of the cDNA which codes for the required protein by conventional methods such as those described by G. Winter *et al* in Nature
15 1982, 299, 756-758 or by Zoller and Smith 1982; Nucl. Acids Res., 10, 6487-6500, or deletion mutagenesis such as described by Chan and Smith in Nucl. Acids Res., 1984, 12, 2407-2419 or by G. Winter *et al* in Biochem. Soc. Trans., 1984; 12, 224-225 or polymerase chain reaction such as described by Mikaelian and Sergeant in Nucleic Acids Research, 1992, 20, 376.

20 When used herein 'compound of the invention' or 'compounds of the invention' relates to the above mentioned chimera.

In a further aspect, the invention provides a process for preparing a compound according to the invention which process comprises expressing DNA encoding said compound in a recombinant host cell and recovering the product.

25 The DNA polymer comprising a nucleotide sequence that encodes the compound also forms part of the invention.

The process of the invention may be performed by conventional recombinant techniques such as described in Maniatis *et. al.*, Molecular Cloning - A Laboratory Manual; Cold Spring Harbor, 1982 and DNA Cloning vols I, II and III (D.M. Glover ed.,
30 IRL Press Ltd).

In particular, the process may comprise the steps of:

- i) preparing a replicable expression vector capable, in a host cell, of expressing a DNA polymer comprising a nucleotide sequence that encodes said compound;
- ii) transforming a host cell with said vector;
- 35 iii) culturing said transformed host cell under conditions permitting expression of said DNA polymer to produce said compound; and
- iv) recovering said compound.

The invention also provides a process for preparing the DNA polymer by the condensation of appropriate mono-, di- or oligomeric nucleotide units.

The preparation may be carried out chemically, enzymatically, or by a combination of the two methods, *in vitro* or *in vivo* as appropriate. Thus, the DNA polymer may be prepared by the enzymatic ligation of appropriate DNA fragments, by
5 conventional methods such as those described by D. M. Roberts *et al* in Biochemistry 1985, 24, 5090-5098.

The DNA fragments may be obtained by digestion of DNA containing the required sequences of nucleotides with appropriate restriction enzymes, by chemical
10 synthesis, by enzymatic polymerisation on DNA or RNA templates, or by a combination of these methods.

Digestion with restriction enzymes may be performed in an appropriate buffer at a temperature of 20°-70°C, generally in a volume of 50µl or less with 0.1-10µg DNA.

Enzymatic polymerisation of DNA may be carried out *in vitro* using a DNA
15 polymerase such as DNA polymerase I (Klenow fragment) in an appropriate buffer containing the nucleoside triphosphates dATP, dCTP, dGTP and dTTP as required at a temperature of 10°-37°C, generally in a volume of 50µl or less.

Enzymatic ligation of DNA fragments may be carried out using a DNA ligase such as T4 DNA ligase in an appropriate buffer at a temperature of 4°C to ambient,
20 generally in a volume of 50µl or less.

The chemical synthesis of the DNA polymer or fragments may be carried out by conventional phosphotriester, phosphite or phosphoramidite chemistry, using solid phase techniques such as those described in 'Chemical and Enzymatic Synthesis of Gene
25 Fragments - A Laboratory Manual' (ed. H.G. Gassen and A. Lang), Verlag Chemie, Weinheim (1982), or in other scientific publications, for example M.J. Gait, H.W.D. Matthes, M. Singh, B.S. Sproat, and R.C. Titmas, Nucleic Acids Research, 1982, 10, 6243; B.S. Sproat and W. Bannwarth, Tetrahedron Letters, 1983, 24, 5771; M.D. Matteucci and M.H. Caruthers, Tetrahedron Letters, 1980, 21, 719; M.D. Matteucci and M.H. Caruthers, Journal of the American Chemical Society, 1981, 103, 3185; S.P.
30 Adams *et al.*, Journal of the American Chemical Society, 1983, 105, 661; N.D. Sinha, J. Biernat, J. McMannus, and H. Koester, Nucleic Acids Research, 1984, 12, 4539; and H.W.D. Matthes *et al.*, EMBO Journal, 1984, 3, 801. Preferably an automated DNA synthesizer is employed.

The DNA polymer is preferably prepared by ligating two or more DNA
35 molecules which together comprise a DNA sequence encoding the compound. A particular process in accordance with the invention comprises ligating a first DNA

molecule encoding a said leptin or variant and a second DNA molecule encoding a said immunoglobulin domain or fragment thereof.

The DNA molecules may be obtained by the digestion with suitable restriction enzymes of vectors carrying the required coding sequences or by use of polymerase chain
5 reaction technology.

The precise structure of the DNA molecules and the way in which they are obtained depends upon the structure of the desired product. The design of a suitable strategy for the construction of the DNA molecule coding for the compound is a routine matter for the skilled worker in the art.

10 The expression of the DNA polymer encoding the compound in a recombinant host cell may be carried out by means of a replicable expression vector capable, in the host cell, of expressing the DNA polymer. The expression vector is novel and also forms part of the invention.

The replicable expression vector may be prepared in accordance with the
15 invention, by cleaving a vector compatible with the host cell to provide a linear DNA segment having an intact replicon, and combining said linear segment with one or more DNA molecules which, together with said linear segment, encode the compound, under ligating conditions.

The ligation of the linear segment and more than one DNA molecule may be
20 carried out simultaneously or sequentially as desired.

Thus, the DNA polymer may be preformed or formed during the construction of the vector, as desired.

The choice of vector will be determined in part by the host cell, which may be prokaryotic, such as *E. coli*, or eukaryotic, such as mouse C127, mouse myeloma,
25 chinese hamster ovary, Cos1 or Hela cells, fungi e.g. filamentous fungi or unicellular yeast or an insect cell such as *Drosophila*. The host cell may also be a transgenic animal.

A preferred host cell is Cos1.

Suitable vectors include plasmids, bacteriophages, cosmids and recombinant
viruses from, for example, baculoviruses, vaccinia or Semliki Forest virus.

30 The preparation of the replicable expression vector may be carried out conventionally with appropriate enzymes for restriction, polymerisation and ligation of the DNA, by procedures described in, for example, Maniatis *et al.*, cited above. Polymerisation and ligation may be performed as described above for the preparation of the DNA polymer. Digestion with restriction enzymes may be performed in an
35 appropriate buffer at a temperature of 20°-70°C, generally in a volume of 50µl or less with 0.1-10µg DNA.

The recombinant host cell is prepared, in accordance with the invention, by transforming a host cell with a replicable expression vector of the invention under transforming conditions. Suitable transforming conditions are conventional and are described in, for example, Maniatis *et al.*, cited above, or "DNA Cloning" Vol. II, D.M. Glover ed., IRL Press Ltd, 1985.

The choice of transforming conditions is determined by the host cell. Thus, a bacterial host such as *E. coli* may be treated with a solution of CaCl_2 (Cohen *et al.* Proc. Nat. Acad. Sci., 1973, 69, 2110) or with a solution comprising a mixture of RbCl , MnCl_2 , potassium acetate and glycerol, and then with 3-[N-morpholino]-propane-sulphonic acid, RbCl and glycerol. Mammalian cells in culture may be transformed by calcium co-precipitation of the vector DNA onto the cells.

The invention also extends to a host cell transformed or transfected with a replicable expression vector of the invention.

Culturing the transformed host cell under conditions permitting expression of the DNA polymer is carried out conventionally, as described in, for example, Maniatis *et al* and "DNA Cloning" cited above. Thus, preferably the cell is supplied with nutrient and cultured at a temperature below 45°C .

The expression product is recovered by conventional methods according to the host cell. Thus, where the host cell is bacterial, such as *E. coli* it may be lysed physically, chemically or enzymatically and the protein product isolated from the resulting lysate. If the product is to be secreted from the bacterial cell it may be recovered from the periplasmic space or the nutrient medium. Where the host cell is mammalian, the product may generally be isolated from the nutrient medium.

The DNA polymer may be assembled into vectors designed for isolation of stable transformed mammalian cell lines expressing the product; e.g. bovine papillomavirus vectors or amplified vectors in chinese hamster ovary cells (DNA cloning Vol.II D.M. Glover ed. IRL Press 1985; Kaufman, R.J. *et al.*, Molecular and Cellular Biology 5, 1750-1759, 1985; Pavlakis G.N. and Hamer, D.H., Proceedings of the National Academy of Sciences (USA) 80, 397-401, 1983; Goeddel, D.V. *et al.*, European Patent Application No. 0093619, 1983).

The activity of the chimeric leptin is determined by injecting it intraperitoneally, intravenously or subcutaneously into test animals such as rodents, for example mice or rats, or primates, for example rhesus monkeys. In order to maximise activity, the test animals are preferably overweight or obese animals that have been made overweight by feeding them on a high fat or other palatable diet, or have acquired fat through the ageing process. In the case of mice, however, the ideal strain is the genetically obese (ob/ob) mouse. The effect of the active compound is seen as a reduction in food intake or increase

in metabolic rate or oxygen consumption. Multiple injections of the active compound - at most twice daily - over a period of a week for rodents or a month for primates, also cause a reduction in body weight and in the size of discrete adipose tissue depots.

Clearance rates are determined by conventional plasma assay using ob-antibodies, for example ELISA methodology.

As indicated above the compounds of the present invention have useful pharmaceutical properties, in particular anti obesity activity and also for the treatment of diseases associated with obesity, such as atherosclerosis, hypertension and, especially, Type II diabetes.

In use the compound will normally be employed in the form of a pharmaceutical composition in association with a human pharmaceutical carrier, diluent and/or excipient, although the exact form of the composition will depend on the mode of administration.

The active compound may be formulated for administration by any suitable route and is preferably in unit dosage form. Advantageously, the composition is suitable for oral, rectal, topical, parenteral, intravenous or intramuscular administration or through the respiratory tract. Preparations may be designed to give slow release of the active ingredient.

The compositions of the invention may be in the form of tablets, capsules, sachets, vials, powders, granules, lozenges, suppositories, reconstitutable powders, or liquid preparations such as oral or sterile parenteral solutions or suspensions. Topical formulations are also envisaged where appropriate.

The invention therefore further provides a pharmaceutical composition comprising a compound of the invention and a pharmaceutically acceptable carrier.

The dosage ranges for administration of the compounds of the present invention are those to produce the desired therapeutic effect. Dosage will generally vary with age, extent or severity of the medical condition and contraindications, if any. For example in the treatment of obesity the unit dosage can vary from less than 1mg to 300mg, but typically will be in the region of 1 to 20mg per dose, in one or more doses, such as one to six doses per day, so that the daily dosage is in the range 0.02-40mg/kg.

Dosages and compositions for the treatment of diseases associated with obesity such as atherosclerosis, hypertension and, especially, Type II diabetes are selected from an equivalent range to that used in the treatment of obesity.

Compositions suitable for injection may be in the form of solutions, suspensions or emulsions, or dry powders which are dissolved or suspended in a suitable vehicle prior to use.

Fluid unit dosage forms are prepared utilising the compound and a pyrogen-free sterile vehicle. The compound, depending on the vehicle and concentration used, can be

either dissolved or suspended in the vehicle. Solutions may be used for all forms of parenteral administration, and are particularly used for intravenous infection. In preparing solutions the compound can be dissolved in the vehicle, the solution being made isotonic if necessary by addition of sodium chloride and sterilised by filtration
5 through a sterile filter using aseptic techniques before filling into suitable sterile vials or ampoules and sealing. Alternatively, if solution stability is adequate, the solution in its sealed containers may be sterilised by autoclaving. Advantageously additives such as buffering, solubilising, stabilising, preservative or bactericidal, suspending or emulsifying agents and/or local anaesthetic agents may be dissolved in the vehicle.

10 Dry powders which are dissolved or suspended in a suitable vehicle prior to use may be prepared by filling pre-sterilised drug substance and other ingredients into a sterile container using aseptic technique in a sterile area. Alternatively the drug and other ingredients may be dissolved in an aqueous vehicle, the solution is sterilised by filtration and distributed into suitable containers using aseptic technique in a sterile area. The
15 product is then freeze dried and the containers are sealed aseptically.

Parenteral suspensions, suitable for intramuscular, subcutaneous or intradermal injection, are prepared in substantially the same manner, except that the sterile compound is suspended in the sterile vehicle, instead of being dissolved and sterilisation cannot be accomplished by filtration. The compound may be isolated in a sterile state or
20 alternatively it may be sterilised after isolation, e.g. by gamma irradiation. Advantageously, a suspending agent for example polyvinylpyrrolidone is included in the composition to facilitate uniform distribution of the compound.

Compositions suitable for administration via the respiratory tract include aerosols, nebulisable solutions or microfine powders for insufflation. In the latter case, particle
25 size of less than 50 microns, especially less than 10 microns, is preferred. Such compositions may be made up in a conventional manner and employed in conjunction with conventional administration devices.

In a further aspect there is provided a method of treating obesity or diseases associated with obesity, such as atherosclerosis, hypertension and, especially, Type II
30 diabetes, in human or non-human mammals which comprises administering to the sufferer an effective, non-toxic amount of a compound of the invention.

Suitable non-human mammals are domestic mammals such as dogs and cats.

The invention further provides a compound of the invention for use as an active therapeutic substance, in particular for use in treating obesity or diseases associated with
35 obesity, such as atherosclerosis, hypertension and, especially, Type II diabetes.

The invention also provides the use of a compound of the invention in the manufacture of a medicament for treating obesity or diseases associated with obesity, such as atherosclerosis, hypertension and, especially, Type II diabetes.

As indicated above the invention also encompasses cosmetic treatments.

5 Accordingly, there is also provided a compound of the invention for use in the cosmetic treatment of human or non-human mammals.

There is also provided a method for the cosmetic treatment of a human or non-human mammal, which treatment comprises administering an effective, non-toxic amount of a compound of the invention to a human or non-human mammal in need
10 thereof.

Cosmetic treatment suitably includes treatment for the improvement of body appearance, such as weight reduction treatment.

The invention also extends to a cosmetic composition, comprising a compound of the invention and a carrier therefor.

15 Compositions of the invention including cosmetic compositions are formulated using known methods, for example those described in standard text books of pharmaceuticals and cosmetics, such as Harry's Cosmeticology published by Leonard Hill Books, Remington's Pharmaceutical Sciences, the British and US Pharmacopoeias.

No unexpected toxicological effects are expected when compounds of the
20 invention are administered in accordance with the present invention.

The following Examples illustrate the invention but do not limit it in any way.

Example 1.**Construction of DNA coding for fusion protein leptin 1-167/IgG4 hinge-CH2-CH3**

The gene coding for a fusion protein comprising human leptin and the
 5 hinge-CH2-CH3 region of human IgG4 is created by recombinant DNA technology, preferably by a two-step recombinant PCR method.

The human 'ob' gene has been prepared synthetically based on the amino acid sequence of Zhang et al. and assembled in the pcDNA3 vector.

The cDNA encoding full length human leptin, nucleotides 1-501 is joined at the
 10 3' end to the 5' end of the hinge-CH2-CH3 region of the cDNA coding for the human IgG4 protein, shown as nucleotides 502-1188 in the DNA sequence below. (Table 1.)

The encoded protein sequence of the leptin/IgG4 chimera is given in Table 2. Leptin 1-167 (numbering as Y. Zhang, R. Proenca, M. Maffei, M. Barone, L. Leopold & J. Friedman. Nature 372:425-432), and IgG4 hinge-CH2-CH3 168-396 (*sequence as*
 15 *Kabat*).

The fusion protein was expressed transiently in Cos1 cells using the pCDN vector system, as described in International Patent Application Publication number WO 96/04388. The mature protein was exported from the cells into the culture medium and was detected by anti-leptin antibody. It was shown to have a size consistent with the predicted structure by
 20 Western blotting analysis under both reducing and nonreducing conditions.

Table 1. DNA sequence of ob/IgG4 chimera, 1188bp

```

25  ATGCATTGGGGAACCCTGTGCGGATTCTTGTGGCTTTGGCCCTATCTTTTCTATGTCCAA
                                           60
    GCTGTGCCCATCCAAAAAGTCCAAGATGACACCAAACCCTCATCAAGACAATTGTCACC
                                           120
30  AGGATCAATGACATTTACACACGCGAGTCAGTCTCCTCCAAACAGAAAGTCACCGGTTTG
                                           180
    GACTTCATTCTGGGCTCCACCCCATCCTGACCCTGTCCAAGATGGACCAGACACTGGCA
35  240
    GTCTACCAACAGATCCTCACATCGATGCCTTCCAGAAACGTCATCCAAATATCCAACGAC
                                           300
40  CTGGAGAACCTCCGGGATCTTCTTCACGTGCTGGCCTTCTCTAAGAGCTGCCACTTGCCC
                                           360
    TGGGCCAGTGGCCTGGAGACCTTGGACAGCCTGGGGGGTGTCTCGAGGCTTCAGGCTAC
                                           420
45  TCCACAGAGGTGGTGGCCCTGAGCAGGCTGCAGGGGTCTCTGCAGGACATGCTGTGGCAG
                                           480
    CTGGACCTCAGCCCCGGGTGCGAGTCCAAATATGGTCCCCCATGCCCATCATGCCAGCA
50  540
  
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CCTGAATTTCTGGGGGGACCATCAGTCTTCCTGTTCCCCC AAAACCCAAGGACACTCTC
 600
 5 ATGATCTCCCGGACCCCTGAGGTCACGTGCGTGGTGGTGGACGTGAGCCAGGAAGACCCC
 660
 GAGGTCCAGTTCAACTGGTACGTGGATGGCGTGGAGGTGCATAATGCCAAGACAAAGCCG
 720
 10 CGGGAGGAGCAGTTCAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAG
 780
 GACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGGCCTCCCGTCATCG
 840
 15 ATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAGCCACAGGTGTACACCCTG
 900
 20 CCCCCATCCCAGGAGGAGATGACCAAGAACCAGGTGAGCCTGACCTGCCTGGTCAAAGGC
 960
 TTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACA ACTAC
 1020
 25 AAGACCACGCCTCCCGTGCTGGACTCCGACGGATCCTTCTTCTCTACAGCAGGCTAACC
 1080
 GTGGACAAGAGCAGGTGGCAGGAGGGGAATGTCTTCTCATGCTCCGTGATGCATGAGGCT
 1140
 30 CTGCACAACCACTACACACAGAAGAGCCTCTCCCTGTCTCTGGGTAAA
 1188
 35

Table 2. Amino acid sequence of leptin/IgG4 chimera, 396aa

40 1 MHWGTL CGFL WLWPYLEYVQ AVPIQKVQDD TKTLIKTIVT
 RINDISE
 51 VSSKQKV TGL DFIPGLHPIL TSKMDQTLA VYQQILTSMP SRNVIQISND
 45 101 LENLRDLLHV LAFSKSCHLP WASGLET LDS LGGVLEASGY
 STEVVALSRL
 151 QGSLQDMLWQ LDLSPGCE SK YGPPCPSCPA PEFLGGPSVF
 LFPPKPKDTL
 50 201 MISRTPEVTC VVVDVSQEDP EVQFNWYVDG VEVHNAKTKP
 REEQFNSTYR

251 VVSVLTVLHQ DWLNGKEYK CKVSNKGLPSS IEKTISKAKG
QPREPVYTL

301 PPSQEEMTKN QVSLTCLVKG FYPSDIAVEW ESNGQPENNY
5 KTTTPVLDSD

351 GSFFLYSRLT VDKSRWQEGN VFSCSVMHEA LHNHYTQKSL SLSLGK

Example 2.**Construction of DNA coding for fusion protein ob 1-167/IgG4 hinge-CH2-CH3 PE variant**

- 5 The gene coding for a fusion protein comprising the human 'ob' protein and the Hinge-CH2-CH3 region of human IgG4 PE (a form of IgG4 mutated as below) is created by recombinant DNA technology, preferably by a two-step recombinant PCR method.

- 10 The cDNA coding for the complete human leptin, amino acids 1-167 (numbering as Y. Zhang, R. Proenca, M. Maffei, M. Barone, L. Leopold & J. Friedman. Nature 372: 425-432) is joined at the 3' end to the 5' end of the hinge-CH2-CH3 region of the cDNA coding for the human IgG4 (PE variant) protein, shown as amino acids 168-396 in the protein sequence below.

- 15 The human 'ob' gene has been prepared synthetically based on the amino acid sequence of Zhang et al, and assembled in the pcDNA3 vector. The encoded protein sequence is given in Table 2.

- 20 Human IgG4 heavy chain PE variant. In IgG4 PE, residue 10 of the hinge (residue 241, Kabat numbering) is altered from serine (S) in the wild type to proline (P) and residue 5 of CH2 (residue 248, Kabat numbering) is altered from leucine (L) in the wild type to glutamate (E). Angal S., King D.J., Bodmer M.W., Turner A., Lawson A.D.G., Roberts G., Pedley B. and Adair R., Molecular Immunology vol30pp105-108, 1993, describe an IgG4 molecule where residue 241 (Kabat numbering) is altered from serine to proline. This change increases the serum half-life of the IgG4 molecule.

- 25 The IgG4 PE variant was created using PCR mutagenesis on the synthetic human IgG4 heavy chain cDNA. The sequence of the IgG4 PE variant is described in Table 1. The residues of the IgG4 nucleotide sequence which were altered to make the PE variant are as follows:
referring to Table 1:

- 30 residue 322 has been altered to "C" in the PE variant from "T" in the wild type;
residue 331 has been altered to "G" in the PE variant from "A" in the wild type; and
residues 343-344 have been altered to "GA" in the PE variant from "CT" in the wild type.

- 35 The fusion protein was expressed transiently in Cos1 cells using the pCDN vector system, as described in International Patent Application Publication number WO 96/04388. The mature protein was exported from the cells into the culture medium and was detected by anti-leptin antibody. It was shown to have a size consistent with the predicted structure by
40 Western blotting analysis under both reducing and nonreducing conditions.

Table 3. DNA sequence of IgG4 PE variant, 984bp

5 SEQ ID No:1
 GCTAGTACCAAGGGCCCATCCGTCTTCCCCCTGGCGCCCTGCTCCAGGAGCACCTCCGAG 60
 AGCACgGCCGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTG 120
 10 TGGAAGTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCTCTCA 180
 GGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACGAAGACC 240
 15 TACACCTGCAACGTAGATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGTCC 300
 AAATATGGTCCCCCATGCCCAcCATGCCCAGCgCCTGAaTTtgaGGGGGGACCATCAGTC 360
 TTCCTGTTCCCCCAAAACCCAAGGACACTCTCATGATCTCCCGGACCCCTGAGGTCACG 420
 20 TGCGTGGTGGTGGACGTGAGCCAGGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGAT 480
 GGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTTCAACAGCACGTAC 540
 25 CGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAG 600
 TGCAAGGTCTCCAACAAAGGCCTCCCGTCaTCgATCGAGAAAACCATCTCCAAGCCAAA 660
 GGGCAGCCCCGAGAGCCACAGGTGTACACCCTGCCCCCATCCCAGGAGGAGATGACCAAG 720
 30 AACCAGGTACGCCTGACCTGCCTGGTCAAAGGCTTCTACCCCAGCGACATCGCCGTGGAG 780
 TGGGAGAGCAATGGGCAGCCGGAGAACAATAACAAGACCACGCCTCCCGTGCTGGACTCC 840
 35 GACGGaTCCTTCTTCTCTACAGCAGGCTAACCGTGGACAAGAGCAGGTGGCAGGAGGGG 900
 AATGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACACAGAAGAGC 960
 CTCTCCCTGTCTCTGGGTAAATGA 984
 40

Table 3A: DNA sequence of ob/IgG4PE chimera, 1188bp

45 ATGCATTGGGGAACCCTGTGCGGATTCTTGTGGCTTGGCCCTATCTTTTCTATGTCCAA 60
 GCTGTGCCCATCCAAAAGTCCAAGATGACACCAAAACCCTCATCAAGACAATTGTCACC 120
 AGGATCAATGACATTTACACACGCAGTCAGTCTCCTCCAACAGAAAGTCACCGGTTTG 180
 50 GACTTCATTCTTGGGCTCCACCCCATCCTGACCCTGTCCAAGATGGACCAGACACTGGCA 240
 GTCTACCAACAGATCCTCACATCGATGCCTTCCAGAAACGTGATCCAAATATCCAACGAC 300

CTGGAGAACCTCCGGGATCTTCTTCACGTGCTGGCCTTCTCTAAGAGCTGCCACTTGCCC
 360
 TGGGCCAGTGGCCTGGAGACCTTGGACAGCCTGGGGGGTGTCTCGAGGCTTCAGGCTAC
 420
 5 TCCACAGAGGTGGTGGCCCTGAGCAGGCTGCAGGGGTCTCTGCAGGACATGCTGTGGCAG
 480
 CTGGACCTCAGCCCCGGGTGCGAGTCCAAATATGGTCCCCCATGCCCAcCATGCCCAGCg
 540
 10 CCTGAATTTGAGGGGGGACCATCAGTCTTCCTGTTCCCCC AAAACCCAAGGACACTCTC
 600
 ATGATCTCCCGGACCCCTGAGGTCACGTGCGTGGTGGTGGACGTGAGCCAGGAAGACCCC
 660
 GAGGTCCAGTTCAACTGGTACGTGGATGGCGTGGAGGTGCATAATGCCAAGACAAAGCCG
 720
 15 CGGGAGGAGCAGTTCAACAGCACGTACCGTGTGGTCAGCGTCTCACCCTCCTGCACCAG
 780
 GACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGGCCTCCCGTCATCG
 840
 20 ATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAGCCACAGGTGTACACCCTG
 900
 CCCCCATCCCAGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGC
 960
 TTCTACCCCAGCGACATCGCCGTGGAGTTGGAGAGCAATGGGCAGCCGGAGAACAACACTAC
 1020
 25 AAGACCACGCCTCCCGTGCTGGACTCCGACGGATCCTTCTTCTCTACAGCAGGCTAACC
 1080
 GTGGACAAGAGCAGGTGGCAGGAGGGGAATGTCTTCTCATGCTCCGTGATGCATGAGGCT
 1140
 30 CTGCACAACCACTACACACAGAAGAGCCTCTCCCTGTCTCTGGGTAAA
 1188

Table 4: Amino acid sequence of ob 1-167/IgG4 hinge-CH2-CH3 PE variant chimera 396aa

35

SEQ ID No: 2

1 MHWGTLCGFL WLWPYLFYVQ AVPIQKVQDD TKTLIKTIVT RINDISHTQS
 40 51 VSSKQKVTGL DFIPGLHPIL TLSKMDQTI A VGGOT YMP SRNVIQISND
 101 LENLRDLLHV LAFSKSCHLP WASGLETLDL LGGVLEASGY STEVVALSRL
 151 QGSLQDMLWQ LDLSPGCESK YGPPCPPCPA PEFEGGPSVF LFPPKPKDTL
 45 201 MISRTPEVTC VVVDVSQEDP EVQFNWYVDG VEVHNAKTKP REEQFNSTYR
 251 VVSVLTVLHQ DWLNGKEYKC KVSNGKLPSS IEKTISKAKG QPREPQVYTL
 50 301 PPSQEEMTKN QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTHPPVLDSD

351 GSFFLYSRLT VDKSRWQEGN VFSCSVMHEA LHNHYTQKSL SLSLGK

Example 3.

- 5 **Construction of DNA coding for fusion protein leptin 1-167/IgG1 hinge-CH2-CH3**
The gene coding for a fusion protein comprising human leptin and the hinge-CH2-CH3 region of human IgG1 is created by recombinant DNA technology, preferably by a two-step recombinant PCR method.

10 The human 'ob' gene has been prepared synthetically based on the amino acid sequence of Zhang et al, and assembled in the pcDNA3 vector.

The cDNA encoding full length human leptin, nucleotides 1-501 is joined at the 3' end to the 5' end of the hinge-CH2-CH3 region of the cDNA coding for the human IgG1 protein, shown as nucleotides 502-1197 in the DNA sequence below. (Table 1.)

15 The encoded protein sequence of the leptin/IgG1 chimera is given in Table 2. Leptin 1-167 (numbering as Y. Zhang, R. Proenca, M. Maffei, M. Barone, L. Leopold & J. Friedman. Nature 372: 425-432) and IgG1 hinge-CH2-CH3 shown as amino acids 168-399.

20 The gene coding for the human IgG1 contains a number of nucleotide substitutions compared to the IgG1 molecule described by Ellison J.W., Berson B.J. and Hood L.E., Nucleic Acids Research vol 10 No. 13 pp4071-4079, 1982. The IgG1 nucleotides which differ from the Ellison J.W. et al published sequence and the resulting amino acid substitutions are as follows (nucleotide numbering as in table 1)

nucleotide 513 is "G" in this variant compared to "T" in the Ellison et al sequence (silent mutation)

25

nucleotides 514-516 are "GCC" in this variant compared to "TGT" in the Ellison et al sequence (resulting in substitution of Ala for Cys in this variant, amino acid 172 in table 2)

30

nucleotide 759 is "T" in this variant compared to "G" in the Ellison et al sequence (silent mutation)

nucleotide 924 is "G" in this variant compared to "T" in the Ellison et al sequence (resulting in substitution of Glu for Asp in this variant, amino acid 308 in table2)

35

nucleotide 928 is "A" in this variant compared to "C" in the Ellison et al sequence (resulting in substitution of Met for Val in this variant, amino acid 310 in table 2)

40

nucleotide 1077 is "T" in this variant compared to "C" in the Ellison et al sequence (silent mutation)

nucleotide 1197 is "G" in this variant compared to "A" in the Ellison et al sequence (silent mutation)

45 The fusion protein was expressed transiently in Cos1 cells using the pCDN vector system, as described in International Patent Application Publication number WO 96/04388.

The mature protein was exported from the cells into the culture medium and was detected by anti-leptin antibody. It was shown to have a size consistent with the predicted structure by Western blotting analysis under both reducing and nonreducing conditions.

5

Table 5. DNA sequence of ob/IgG1 chimera 1197bp

ATGCATTGGGGAACCCGTGTGCGGATTCTTGTGGCTTTGGCCCTATCTTTTCTATGTCCAA
60

10 GCTGTGCCCATCCAAAAAGTCCAAGATGACACCAAAACCCTCATCAAGACAATTGTCACC
120

AGGATCAATGACATTTACACACGCAGTCAGTCTCCTCCAAACAGAAAGTCACCGGTTTG
15 180

GACTTCATTTCCTGGGCTCCACCCCATCCTGACCCTGTCCAAGATGGACCAGACACTGGCA
240

20 GTCTACCAACAGATCCTCACATCGATGCCTTCCAGAAACGTGATCCAAATATCCAACGAC
300

CTGGAGAACCTCCGGGATCTTCTTCACGTGCTGGCCTTCTCTAAGAGCTGCCACTTGCCC
25 360

TGGGCCAGTGGCCTGGAGACCTTGGACAGCCTGGGGGGTGTCTCGAGGCTTCAGGCTAC
420

30 TCCACAGAGGTGGTGGCCCTGAGCAGGCTGCAGGGGTCTCTGCAGGACATGCTGTGGCAG
480

CTGGACCTCAGCCCCGGGTGCGAGCCCAAATCGGCCGACAAACTCACACATGCCACCG
540

35 TGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTCCCCCCTAAACCCAAG
600

GACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCAC
40 660

GAAGACCCTGAGGTCAAGTTCAACTGGTACGTGACGGCGTGGAGGTGCATAATGCCAAG
720

45 ACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTC
780

CTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTC
840

50 CCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTG
900

TACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTACGCTGACCTGCCG
960

GTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAG
1020

5 AACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCTCTATAGC
1080

AAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATG
1140

10 CATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAG
1197

15 **Table 6. Amino acid sequence of leptin/IgG1 chimera, 399aa**

1 MHWGTLGFL WLWPYLFYVQ AVPIQKVQDD TKTLIKTIVT RINDISHTQS
51 VSSKQKVTGL DFIPGLHPIL TSKMDQTLA VYQQILTSMP SRNVIQISND
20 101 LENLRDLLHV LAFSKSCHLP WASGLETLDL LGGVLEASGY STEVVALSRL
151 QGSLQDMLWQ LDLSPGCEPK SADKTHTCPP CPAPELLGGP SVFLFPPKPK
25 201 DTLMISRTPE VTCVVVDVSH EDPEVKFNWY VDGVEVHNAK TKPREEQYNS
251 TYRVVSVLTV LHQDWLNGKE YKCKVSNKAL PAPIEKTISK AKGQPREPQ
301 YTLPPSREEM TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTTPVL
30 351 DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ KSLSLSPGK

35

Example 4.

40

**Construction of DNA coding for fusion protein leptin 1-167/IgG1 hinge-CH2-CH3
GT linker variant**

45 The gene coding for a fusion protein comprising human leptin and the
hinge-CH2-CH3 region of human IgG1 with a 'GT' two amino acid linker between
the two parts of the fusion molecule, is created by recombinant DNA technology,
preferably by a two-step recombinant PCR method.

The human 'ob' gene has been prepared synthetically based on the amino acid sequence
of Zhang et al, and assembled in the pcDNA3 vector.

The cDNA encoding the full length human leptin (nucleotides 1-501) is joined at the 3' end to the 5' end of the hinge-CH2-CH3 region of the IgG1 cDNA (nucleotides 508-1203). The two amino acid linker between the two parts of the fusion is encoded by the nucleotide sequence GGTACC (502-507). See Table 1.

5 The encoded protein sequence of the leptin/IgG1(GT) chimera is given in Table 2. Leptin (numbering as Y. Zhang, R. Proenca, M. Maffei, M. Barone, L. Leopold & J. Friedman. Nature 372:425-432), followed by the GT linker (168-169) and IgG1 H-CH2-CH3 170-401.

10 The gene coding for the human IgG1 contains a number of nucleotide substitutions compared to the IgG1 molecule described by Ellison J.W., Berson B.J. and Hood L.E., Nucleic Acids Research vol 10 No. 13 pp4071-4079, 1982. The IgG1 nucleotides which differ from the Ellison J.W. et al published sequence and the resulting amino acid substitutions are as follows (nucleotide numbering as in table 1)

15 nucleotide 519 is "G" in this variant compared to "T" in the Ellison et al sequence (silent mutation)

nucleotides 520-522 are "GCC" in this variant compared to "TGT" in the Ellison et al sequence (resulting in substitution of Ala for Cys in this variant, amino acid 174 in table 2)

20

nucleotide 759 is "T" in this variant compared to "G" in the Ellison et al sequence (silent mutation)

25 nucleotide 924 is "G" in this variant compared to "T" in the Ellison et al sequence (resulting in substitution of Glu for Asp in this variant, amino acid 308 in table 2)

nucleotide 928 is "A" in this variant compared to "C" in the Ellison et al sequence (resulting in substitution of Met for Val in this variant, amino acid 310 in table 2)

30 nucleotide 1077 is "T" in this variant compared to "C" in the Ellison et al sequence (silent mutation)

nucleotide 1197 is "G" in this variant compared to "A" in the Ellison et al sequence (silent mutation)

35

The fusion protein was expressed transiently in Cos1 cells using the pCDN vector system, as described in International Patent Application Publication number WO 96/04388. The mature protein was exported from the cells into the culture medium and was detected by anti-leptin antibody. It was shown to have a size consistent with the predicted structure by Western blotting analysis under both reducing and nonreducing conditions.

40

Table 7. DNA sequence of ob/IgG1'GT' chimera , 1203bp

ATGCATTGGGGAACCCTGTGCGGATTCTTGTGGCTTGGCCCTATCTTTTCTATGTCCAA
60

45

GCTGTGCCCATCCAAAAAGTCCAAGATGACACCAAAACCCCTCATCAAGACAATTGTCAACC
 120
 5 AGGATCAATGACATTTACACACACGCAGTCAGTCTCCTCCAAACAGAAAGTCACCGGTTTG
 180
 GACTTCATTCCTGGGCTCCACCCCATCCTGACCCTGTCCAAGATGGACCAGACACTGGCA
 240
 10 GTCTACCAACAGATCCTCACATCGATGCCTTCCAGAAACGTGATCCAAATATCCAACGAC
 300
 CTGGAGAACCTCCGGGATCTTCTTCACGTGCTGGCCTTCTCTAAGAGCTGCCACTTGCCC
 360
 15 TGGGCCAGTGGCCTGGAGACCTTGGACAGCCTGGGGGGTGTCTCGAGGCTTCAGGCTAC
 420
 TCCACAGAGGTGGTGGCCCTGAGCAGGCTGCAGGGGTCTCTGCAGGACATGCTGTGGCAG
 480
 20 CTGGACCTCAGCCCCGGGTGCGGTACCGAGCCCAAATCGGCCGACAAACTCACACATGC
 540
 25 CCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCTCTTCCCCCAGAAA
 600
 CCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTG
 660
 30 AGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAAT
 720
 GCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTC
 780
 35 ACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAA
 840
 40 GCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCA
 900
 CAGGTGTTCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTGAGCCTGACC
 960
 45 TGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAG
 1020
 CCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTC
 1080
 50 TATAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCC
 1140
 55 GTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGT
 1200

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AAG
1203

5

Table 8. Amino acid sequence of leptin/IgG1 'GT' chimera, 401aa

1 MHWGTLGFL WLWPYLFYVQ AVPIQKVQDD TKTLIKTI VT
10 RINDISHTQS
51 VSSKQKV TGL DFIPGLHPIL TSKMDQTLA VYQQILTSMP SRNVIQISND
101 LENLRDLLHV LAFSKSCHLP WASGLET LDS LGGVLEASGY
15 STEVVALSRL
151 QGSLQDMLWQ LDLSPGCGTE PKSADKTHTC PPCAPELLG
GPSVFLFPPK
20 201 PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN
AKTKPREEQY
251 NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPAPIEKTI
25 SKAKGQPREP
301 QVYTLPPSRE EMTKNQVSLT CLVKGFYPSD IAVEWESNGQ
PENNYKTTTP
351 VLDS DGSFFL YSKLTVDKSR WQQGNVFSCS VMHEALHNHY
30 TQKSLSLSPG
401 K

35

Claims:

1. Chimeric leptin or a chimeric mutant or derivative of leptin.
- 5 2. A chimera according to claim 1, wherein the leptin is human leptin.
3. A chimera according to claim 1 or claim 2, wherein the leptin or a mutant or variant thereof is fused to a human immunoglobulin domain or a mutant or variant thereof.
- 10 4. A chimera according to any one of claims 1 to 3, wherein the chimeric protein comprises one human immunoglobulin domain.
5. A chimera according to claim 4, wherein the human immunoglobulin domain is fused to the C-terminus of leptin.
- 15 6. A chimera according to any one of claims 1 to 4, which comprises a human immunoglobulin Fc domain.
7. A chimera according to claim 6, wherein the human immunoglobulin Fc domain is an IgG4PE variant, an IgG4, IgG1 or an IgG1GT variant, in particular the hinge-CH₂-CH₃ region in each case.
- 20 8. A chimera according to claim 7, wherein the variant is a hinge-CH₂-CH₃ variant.
- 25 9. Chimeric leptin selected from the list consisting of:
leptin 1-167/IgG4 hinge-CH₂-CH₃;
leptin 1-167/IgG4 hinge-CH₂-CH₃ PE variant;
leptin 1-167/IgG1 hinge-CH₂-CH₃; and
leptin 1-167/IgG1 hinge-CH₂-CH₃ GT linker variant.
- 30 10. A process for preparing a chimera according to any one of claims 1 to 8, which process comprises expressing DNA encoding said compound in a recombinant host cell and recovering the product.
- 35 11. A process according to claim 10, which process comprises the steps of:
 - i) preparing a replicable expression vector capable, in a host cell, of expressing a DNA polymer comprising a nucleotide sequence that encodes said chimera;
 - ii) transforming a host cell with said vector;

- iii) culturing said transformed host cell under conditions permitting expression of said DNA polymer to produce said chimera; and
- iv) recovering said chimera.

- 5 12. A DNA polymer comprising a nucleotide sequence that encodes a chimera according to any one of claims 1 to 8.
13. A vector which comprises a DNA polymer according to claim 12.
- 10 14. A host cell transformed or transfected with a DNA polymer according to claim 12 or a vector according to claim 13.
- 15 15. A pharmaceutical composition comprising a chimera as claimed in claim 1 and a pharmaceutically acceptable carrier.
16. A chimera according to claim 1, for use as an active therapeutic substance.
17. A chimera according to claim 1, for use in the treatment of obesity or diseases associated with obesity.
- 20 18. A method for the treatment of obesity or diseases associated with in human or non-human mammal, which method comprises administering to the sufferer an effective, non-toxic amount of a chimera as claimed in claim 1.
- 25 19. A chimera as claimed in claim 1, for use in the cosmetic treatment of human or non-human mammals.
20. A method for the cosmetic treatment of a human or non-human mammal, which treatment comprises administering an effective, non-toxic amount of a compound of the invention to a human or non-human mammal in need thereof.
- 30

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification⁶ : C12N 15/12, 15/85, 5/10, C07K 14/47, A61K 38/17	A3	(11) International Publication Number: WO 97/00319 (43) International Publication Date: 3 January 1997 (03.01.97)
(21) International Application Number: PCT/GB96/01388 (22) International Filing Date: 11 June 1996 (11.06.96) (30) Priority Data: 9511935.0 13 June 1995 (13.06.95) GB (71) Applicant (for all designated States except US): SMITHKLINE BEECHAM PLC (GB/GB); New Horizons Court, Brentford, Middlesex TW8 9EP (GB). (72) Inventors; and (75) Inventors/Applicants (for US only): BROWNE, Michael, Joseph (GB/GB); (GB). CHAPMAN, Conrad, Gerald (GB/GB); (GB). CLINKENBEARD, Helen, Elizabeth (GB/GB); (GB). ROBINSON, Jeffrey, Hugh (GB/GB); SmithKline Beecham Pharmaceuticals, Coldharbour Road, The Pinnacles, Harlow, Essex CM19 5AD (GB). (74) Agent: RUTTER, Keith; SmithKline Beecham, Corporate In- tellectual Property, SB House, Great West Road, Brentford, Middlesex TW8 9BD (GB).		(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the</i> <i>claims and to be republished in the event of the receipt of</i> <i>amendments.</i> (88) Date of publication of the international search report: 10 April 1997 (10.04.97)
(54) Title: CHIMERIC LEPTIN FUSED TO IMMUNOGLOBULIN DOMAIN AND USE (57) Abstract <p>Chimeric leptin which are proteins comprising leptin or a mutant or a variant thereof fused to a human immunoglobulin domain. One favoured immunoglobulin domain is the human immunoglobulin Fc domain. The chimeric derivatives of leptin have, despite their large molecular size, good pharmacological activity combined with prolonged clearance rates. These derivatives of leptin are therefore indicated to be particularly useful for the treatment or prophylaxis of obesity or diseases and conditions associated with obesity such as atherosclerosis, hypertension and type II diabetes.</p>		

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 96/01388

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C12N15/85 C12N5/10 C07K14/47 A61K38/17

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	WO 96 35787 A (CHIRON CORP) 14 November 1996 see claims; example 5 ---	1,2, 10-14
E	WO 96 34885 A (SMITHKLINE BEECHAM PLC ;SMITH RICHARD ANTHONY GODWIN (GB); BEELEY) 7 November 1996 see claims; examples ---	1,2
P,A	WO 96 05309 A (UNIV ROCKEFELLER ;FRIEDMAN JEFFREY M (US); ZHANG YIYING (US); PROE) 22 February 1996 see page 43, line 18 - page 46, line 14; claims; examples --- -/--	1,18-20

☒ Further documents were searched. ☐ Patent family members are listed in annex.

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Date of the actual completion of the international search

21 February 1997

Date of mailing of the international search report

06.03.97

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Fuhr, C

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>NATURE, vol. 372, no. 6505, 1 December 1994, pages 425-432, XP000602062 YIYING ZHANG ET AL: "POSITIONAL CLONING OF THE MOUSE OBESE GENE AND ITS HUMAN HOMOLOGUE" cited in the application see the whole document</p> <p style="text-align: center;">---</p>	1,18
A	<p>EP 0 464 533 A (BEHRINGWERKE AG ;GEN HOSPITAL CORP (US)) 8 January 1992 cited in the application see claims; examples</p> <p style="text-align: center;">-----</p>	1

INTERNATIONAL SEARCH REPORT

International application No.

PCT/GB 96/01388

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 18
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim 18 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/compositions.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the claims were timely paid by the applicant, this International Search Report covers only the claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 96/01388

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9635787	14-11-96	AU-A- 5737696	29-11-96
WO-A-9634885	07-11-96	NONE	
WO-A-9605309	22-02-96	AU-A- 3329895	07-03-96
		DE-A- 19531931	07-03-96
		GB-A- 2292382	21-02-96
		ZA-A- 9506868	09-04-96
EP-A-0464533	08-01-92	AU-B- 655421	22-12-94
		AU-A- 7935791	02-01-92
		CA-A- 2045869	29-12-91
		JP-A- 5247094	24-09-93

Form PCT.15A/210 (patent family member) (July 1992)